# Effect of Organophosphate Pesticide Residues on Agricultural Workers: Some Alternative Strategies for Evaluating Blood Cholinesterase Changes

Henry P. Anderson, MA, MPH, Berkeley, California

## I. Introduction

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) of 1947, as amended, requires that manufacturers of pesticides moving in interstate commerce apply to the Environmental Protection Agency for "registration". Applicants submit information on chemical composition of a product, pests against which it is considered effective, crops on which it is to be used, recommended strengths and formulations, and the like.

Pesticide safety is not the primary concern of FIFRA. This concern has found its most concrete Federal expression to date in the 1954 "Miller amendment" to the Food, Drug, and Cosmetic Act, calling for the establishment

Submitted for publication, January 5, 1972

From the Community Studies on Pesticides, Bureau of Occupational Health and Environmental Epidemiology, California State Department of Public Health.

Reprint requests to Community Studies on Pesticides, California State Department of Public Health, 2151 Berkeley, Berkeley, CA 94704.

of pesticide residue "tolerances" on raw agricultural commodities.*

Thus, for example, the administrator of the Act may "upon his own initiative or upon the request of any interested person" rule that the allowable residue of some relatively toxic organic phosphorus compound on fresh peaches shall be one part per million. The manufacturer, university-based agricultural experiment stations, and other agencies, conduct the research necessary to translate this tolerance limit into agricultural practice. If the compound breaks down moderately rapidly, a residue of less than one part per million on peaches may be reached in a week. Instructions for use will then contain the warning "Do not apply within 7 days of harvest". The labels on longer-lived organophosphates may include warnings against application within 14, 21, or 30 days of harvest--occasionally even longer. On some very short-lived materials there are no limitations at all on the period between application and re-entry.**

In terms of protecting the American consumer, this system seems to have worked quite well. Although some questions of teratogenicity and other long-term effects have not been fully resolved, few if any overt illnesses from pesticide residues on foodstuffs have been reported since passage of the Miller amendment in 1954.

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* As this is being written, a "Federal Environmental Pesticide Control Act of 1971" is pending in the U.S. Congress. Although this Act would modify FIFRA in various ways, it would not alter any of the arguments basic to the present paper.

** Discussion throughout this paper is limited to problems of research involving organic phosphorus compounds. Problems of carbamates and chlorinated hydrocarbons are, each in their own way, quite different, and call for quite different research designs.

It must be noted, however, that the sole intent of the Miller amendment is protection of the person at the very end of the chain of food production, processing,and distribution: the child who has strawberries on his breakfast cereal, the man who has an apple in his lunch pail, the housewife who serves green salad at dinner. But the consumer is not the only party with a stake in pesticide safety-- indeed, he is not the party with the most direct and serious stake. Persons who come into contact with pesticides or pesticide residues in the course of their employment are likely to be much more heavily exposed than would be possible through consumption of fruits and vegetables.

Occupationally-exposed classes include workers in the factories where pesticides are manufactured, structural pest control operators, formulators and "swampers", crop-dusting pilots and flagmen, and perhaps salesmen and demonstrators. Such workers are covered by general occupational safety statutes, workmen's compensation insurance, and other types of protective legislation and administrative regulations. In California, for example, there is an Industrial Safety Order, promulgated by the state Department of Industrial Relations, which requires that employees "regularly occupied" in the mixing or spraying of organic phosphate pesticides be under the supervision of a licensed physician.

All of this, however, omits from the equation one very large and very directly concerned occupational group: the agricultural laborers who thin and pick fruit, hoe weeds, prune, irrigate, and perform various other tasks in fields, orchards, or vineyards where pesticides have been applied. To the extent that policy-makers have considered farm laborers at all, it has evidently been assumed that they would be adequately protected by regulations designed to protect consumers. For example, if it has been determined that a 14 day waiting period between application and harvest is sufficient to reduce residues "below tolerance", it has been taken for granted that such a waiting period is also, incidentally, sufficient to protect the persons who pick the crop.

There are at least three crucial respects in which any analogy between consumer safety and farm worker safety breaks down. (1) Consumer exposure is almost entirely through ingestion. Farm worker exposure is almost entirely through dermal absorption and inhalation. The latter forms of toxicity cannot be extrapolated from the former. For instance, the oral toxicity of Guthion is rated at 7-18 mg/kg, depending on the researcher, making it rather more toxic than methyl parathion, which is rated at 14-24 mg/kg. However, in terms of dermal toxicity, methyl parathion is by far the more hazardous: in the 60-70 range, compared with something over 200.

(2) Farm worker exposure is largely the result of contact with leaf surfaces, and inhalation of orchard dust; the consumer's exposure is confined to the fruit itself.* Pesticide residues are likely to be more highly

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* Here, and elsewhere throughout this paper, references to "orchards", "fruit workers", and the like, are for ease of expression, and should be taken to include vine crops and certain row crops as well. The problems under discussion here are greatest in tree crops, but are found also in grapes, berries, artichokes, brussels sprouts, etc.

concentrated on foliage than on fruit, and they apparently break down more slowly on leaves, twigs, bark and soil, than on fruit.[1]

(3) By far the gravest shortcoming of any analogy between consumer safety and farm worker safety is the fact that the exposure of a professional handler of fruit is much more prolonged and intense than could possibly be matched by a consumer. There are definite biological limits on how many peaches or pears a person can eat in a day, week, month or lifetime. These limits are taken into account by government agencies in setting tolerances. The tolerance of 10 ppm of phosalone on apples, for example, would obviously have to be much lower if man, like the coddling moth larva, ate at least his body weight in apples each day.

There is no comparable biological limitation to the amount of residue with which a farm worker may come into contact. The number of leaves against which he brushes in a day is limited only by his own energy and expertise. Ironically, and contrary to the pattern in many other areas of industrial safety, the more skilled and dedicated the farm worker, the greater his peril.

It seems a reasonable estimate that a consumer would have to eat several hundred pounds of peaches or oranges per day to match the pesticide "body burden" of the fruit picker who brushes against thousands of leaves and inhales thousands of times in the course of each day's employment. Such estimates must, at the moment, be grossly speculative. Although exhaustive research has been conducted on "market basket" residues, and under the spur of consumer groups more is being conducted all the time, very little has been conducted on the problem of farm worker exposure--there are no special interest groups demanding that it be done and there are no legal requirements that it be done. The manufacturers of pesticides are not required to generate any kind of data bearing directly on farm worker safety as a condition of selling their products in this country.*

As a consequence, agricultural field workers have, since the dawning of the age of organophosphate insecticides, sustained the effects of exposure to residues. There is reason to believe that most of these effects are subacute. They have been likened to the proverbial iceberg, and there are no very satisfactory mechanisms for assessing its true dimensions. In most states, farm laborers are excluded from workmen's compensation insurance. In California, where they are covered, it has been responsibly reported that only a small percentage of the potential cases involving pesticides ever come to light.[2]

On occasion, however, the problem of pesticide residues moves from the realm of the subacute and difficult-to-diagnose to the level of acute poisonings about which there can be no diagnostic doubt. We are not referring here to homicides, suicides and accidents: the leftover parathion kept in a ginger ale bottle, drunk by mistake by an inquisitive child or thirsty worker. We are referring here to "epidemics" of organophosphate poisoning which break out now and then among farm workers--usually fruit pickers--even when all regulations governing the use of the pesticide have been followed scrupulously.

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* Data such as oral toxicity in rats bears only indirectly on farm worker safety. In practical terms, such information translates into recommendations which are platitudinous and almost universally ignored: e.g., harvest workers never have and never will wear rubber protective clothing.

For example, in 1963, nearly a hundred peach pickers in one San Joaquin Valley county developed organic phosphate poisoning, confirmed by laboratory and/or physician examination, although the waiting period required at that time for the compound involved (parathion, 14 days) had been honored in every case.[3]

For another example, there were several incidents in the late spring of 1970, in another San Joaquin Valley county, involving orange pickers. Labels on the products which seemed implicated called for waiting periods of seven days, ranging down to "no time limitations" at all. Yet crew members became ill even when 12 days, and in some cases more, had elapsed between application of the compounds and re-entry into the premises.

As a direct consequence of these 1970 outbreaks, the California Director of Agriculture, within the scope of authority granted him under the state's Agricultural Code, issued regulations which substantially extended the waiting periods for several organic phosphorus materials in citrus and certain other crops. The manufacturers of two of the products in question undertook research designed to demonstrate the adequacy of the old waiting periods (which it failed to do).

A few months later, the Agricultural Extension Service of the University of California tested the same two compounds, plus two others. As of this writing, several other studies of the same general sort are in progress or are planned.

For several reasons, it seems likely that more studies of this type will be called for in the future. Manufacturers whose products have been tested for safe re-entry periods will presumably not be content if their competitors continue to enjoy shorter waiting periods which have not been empirically tested. Conversely, manufacturers whose products have not been tested will probably not be satisfied with that status if objective experiments result in shorter re-entry times for their competitors.* One cannot altogether rule out the possibility that pressure for independent testing will arise from labor groups or others whose motivation is concern for the safety of agricultural workers. Under existing Federal legislation and proposed amendments, it appears that the Administrator of the Environmental Protection Agency could, if he wished, require the positive establishment of re-entry periods as a pre-condition for the registration or re-registration of all economic poisons.

When and if this comes to pass, there will have to be standards for the conduct and interpretation of such research. In the remainder of this paper, we shall examine some of the methodological problems certain to arise in any attempt to establish safe waiting periods for organic phosphorus pesticides in agriculture. Data from the University of California study mentioned above will be used for illustrative purposes. Particular emphasis will be placed on problems of interpreting the dependent variable.** It should be noted that our concern, throughout, is methodological rather than substantive. It is not our purpose, here, to conclude that the waiting period for Compound A should be 21 days, Compound B, 30 days, or combinations of A and B, 45 days. Our purpose is to clarify some of the problems in data-gathering and analysis which are likely to prove troublesome to researchers who are engaged in the actual setting of such minimum re-entry periods.

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* In all cases to date, however, controlled experimentation has pointed toward the necessity for longer rather than shorter re-entry periods.

** Measurement and interpretation of the independent and intervening variables with in a series of papers being prepared for separate publication.

## II. Research Design

The University of California study was representative of a type variously known as the longitudinal, prospective, or time study. When reduced to essentials, any research of this type consists of testing the hypothesis that when intervening variables are held constant, the association of Variable A, known as the independent variable, and Variable B, the dependent variable, will result in a measurable, replicable change (or, as the null hypothesis has it, will fail to produce a significant change).

There are generally accepted statistical conventions in terms of which the investigator may judge whether there is or is not a stable association between A and B. At the 5% level of confidence, for example, there is one chance in twenty that the change observed in the dependent variable was due to the selection of a fortuitous sample and that another sample drawn from the same universe would not yield comparable results. At the 1% level of confidence--a more widely used index of statistical significance--the chances are only one in a hundred that the results obtained were due to sampling factors. Or, to put it the other way, later researchers drawing samples from the same universe and using the same research methods, could expect to obtain comparable results ninety nine times out of a hundred trails.

Since this paper focusses upon the dependent variable, we shall here review only briefly some of the considerations to be borne in mind in handling the dependent variable are dealt.

A. Independent Variable

In studies such as that conducted by the University of California, the independent variable consists of a residue of an organic phosphorus pesticide or combination of pesticides on the fruit, bark, twigs, background dust and particularly the leaves of a particular crop. Following are a few of the principles of research design appropriate to management of this variable.

1. The test plot must be absolutely free from pre-existing residues.
2. Application of the test material must be by skilled personnel, with equipment in good repair, to ensure an even distribution of the material throughout the test plot.
3. A systematic sampling method needs to be devised in advance and followed rigorously throughout the study. Research aides, for example, must be instructed as to precisely how many leaves to pull from each plant, the location (top, bottom, inside, outside), etc. The sample should be designed in such a way that it provides the most accurate possible indication of the true exposure of workers.
4. Analysis should be limited to residues which are actually "available" to workers. There is no point in studying soil a foot below the surface, or the interior of an orange.
5. Analysis should be made of metabolites as well as parent compounds.
6. If more than one compound is tested, sufficient test plots need to be set aside to test each material separately, as well as in combination.
7. The study needs to be conducted over a sufficiently long period, with enough sampling points, to calculate a convincing degradation curve. With a comparatively short-lived material, sampling may need to be

done every day; with a more persistent organophosphate, weekly sampling may be sufficient.

8. The quantity and formulation of pesticide to be applied depends on the objective of the research. An investigator may choose to study the effects of the average or "typical" application. His conclusions regarding a safe re-entry period will then be adequate for the majority of applications of that particular compound in that particular crop. But if there is an unusually heavy infestation, calling for the maximum allowable application, a separate piece of research will be required. Some researchers therefore feel that their time and resources are best spent in testing the compound under extreme conditions: the maximum amount of active material per acre legally permissible; the most hazardous formulation (presumably a wettable powder in a low volume dilutant). Any standards which are set on this basis will be more than ample for less extreme applications.

However, creating the most hazardous conditions likely ever to be encountered in actual practice raises an ethical question: does a researcher working with human subjects have a right to maximize the danger? How can the hazards be brought to a tolerable level? Many ethical problems arise in any study involving pesticides and human participants. The investigator must familiarize himself with these problems and their solutions before he begins.[4]

B. Intervening Variables

In studies of the type under discussion here, management of intervening variables includes such considerations as the following:

1. Every feasible precaution must be taken that participants are not

exposed to organic phosphorus materials outside of the controlled situation. Home use of pesticides should be monitored. Participants should be warned not to wear unlaundered work clothes from the pre-experimental period. Even so simple a matter as eating lunch in a grove across the road from the test grove may have a distorting influence.

2. If a crop activity is of a type performed largely or entirely by men (e.g., cantaloupe harvesting) or largely by women (e.g., field sorting of canning tomatoes), study participants should be selected accordingly.
3. Similarly, the sample should reflect the age spread and ethnic distribution of the persons likely to be found performing the crop activity in real life.
4. The most important demographic characteristic of all, in studies such as this, is customary occupation. It is essential that participants be bona fide farm workers. Recruitment of students might be a great deal easier for a University researcher, but the key intervening variable which must be controlled is rate of movement on the job, for this is the determinant of exposure to the independent variable. One who has not had years of experience could scarcely approach the number of leaves brushed against by a professional fruit picker in the course of an average day's work.
5. In order to duplicate real-life conditions, it is necessary, furthermore, that the farm workers recruited to participate in the study be specialists in the crop involved. A grape picker is not a citrus picker any more than a journeyman carpenter is a journeyman plumber.
6. Again in the interests of reproducing real-life exposure insofar as

possible, workers should be paid on the basis which is customary in the crop involved: usually a piece rate or "incentive" system, which has the practical effect of inducing the worker to press ahead at top speed.*

7. The participants should be representative of their farm worker compatriots in other ways, insofar as feasible and consistent with research ethics. The study must, of course, be explained to all subjects in advance. To that extent, they are "atypical" in the sense that they have knowledge most agricultural laborers do not. But beyond that, it is desirable that the study participants be neither more nor less sophisticated or advantaged than farm workers generally. For example, if it is standard practice that handwashing facilities be provided in orchards, and that fruit pickers use them before eating lunch, then these conditions should be duplicated in the study. If these are not standard practices, then they should not be provided.
8. A thoroughgoing medical history and physical examination must be given all participants in advance, to screen out those with conditions which may affect the dependent variable: acute and chronic liver diseases, dermatomyositis, pulmonary tuberculosis, myocardial infarction, etc.

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* This is not to rule out the possibility that subjects may be paid an additional amount for their time and trouble in having blood specimens drawn, participating in interviews, and other aspects of the study.

C. Dependent Variable

In any study of the effects of pesticide residues on farm workers' health, the dependent variable, self-evidently, is the health status of farm workers. But this is not by any means so simple as it may sound. The researcher must find an index or constellation of indices, which accurately reflects health status; which co-varies systematically with the independent variable; which is objective, quantifiable, and relatively quick and inexpensive to measure; and which does not represent a serious threat to the life or well being of the study participants.* These criteria are not easy to satisfy.

For several reasons, the gross symptoms of organophosphate poisoning are unacceptable as a dependent variable. Such symptoms as headache and nausea are highly subjective. What is painful or uncomfortable to one subject may be accepted stoically by another. Theoretically, a symptom such as headache might be put on a quantitative footing, but the equipment required would be so massive and expensive, and interpretation so difficult that electroencephalography and the like may rule out on practical grounds.

What is more, acute symptoms constitute an effect so advanced they must be rejected for ethical reasons. What is needed is an indicator which does not actually represent poisoning in the usual sense, but an "early warning system"--a physiological precursor of overt symptoms.

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* The time will probably come when re-entry periods can be based simply on knowledge of toxicity and decay curves, without the necessity of human experimentation. It will be sufficient, then to say "Do not enter until leaf residues are below ten parts per million", or whatever. But for the moment, human studies are necessary, since no one, knows the effects of ten parts per million, thirty, five, one or any other leaf residue level.

K.W. Jager and associates have suggested the use of electromyography to detect early impairment of neuromuscular function among persons occupationally exposed to organophosphorus compounds.[5] The method, however, has not to date been tested on agricultural or other workers in the United States.

For the moment, the best available indicator appears to be activity of cholinesterase and pseudocholinesterase enzymes in the blood. Cholinesterase testing has the virtues of precision and objectivity. Among several well-standardized laboratory techniques, the University of California study utilized one which seems particularly sensitive, rapid, and reproducible: the automated titrimetric method commonly known as "pH stat".[6]

Cholinesterase testing is not without intrinsic limitations. In their study of pesticide manufacturing workers, Jager, et al., found many "false negatives": that is, workers adversely affected by exposure to organic phosphates whose blood cholinesterase levels were "normal".[7] Many investigators have demonstrated that "false positives" are perhaps even more common: i.e., cholinesterase activity may be depressed by 25% or more in the absence of exposure to known anticholinesterase substances.[8,9,10]

In addition to these limitations inherent in cholinesterase assays as such, serious methodological problems arise when an attempt is made to study cholinesterase levels among agricultural workers. Among other types of subjects, the research design can be quite straight-forward: a blood test before exposure to organophosphorus residues, a blood test after exposure. If there is a statistically significant difference, it can only be due to the action of the independent variable.

But such comparative simplicity is permissible only if it can be assumed that the study group is in fact unexposed before the study begins. If the study group consists of structural pest control operators, crop-dusting pilots, pesticide manufacturing workers, formulators, salesmen, and the like, the methodology grows more demanding, but is still manageable: instruct the employer to hold participants away from all exposure to cholinesterase inhibitor during a period of several weeks prior to the beginning of the study. Such cooperation is not difficult to obtain , in industry, the research is likely to be not only sanctioned by the employing firm, but supported financially.

To insulate agricultural workers from all organic phosphorus exposure for several weeks, however, is not so readily arranged. Either they must be forbidden to work at all, or some type of non-exposed employment must be found. During the harvest season--the season in which cholinesterase testing is most pertinent--it is out of the question to think any substantial portion of the farm labor force could be shifted to non-harvest activities. There are not that many fences to be painted or tractor tires to be changed. It is equally implausible to imagine that growers, the public, or even the workers themselves would consent to enforced idleness during this period of the year. The expense of maintaining the workers and their families at a decent level of living would be great-- and the value of the unharvested crops would be greater.*

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* Such economic concern cannot be confined to small numbers of study participants. It has been seriously proposed that cholinesterase testing be made a pre-condition of all agricultural employment.

Furthermore, even if it were possible to transfer a cohort of farm workers from harvesting to box making or some other activity, or if it were possible to keep them at home all day, there would still be problems of "moonlighting", drift from orchards adjacent to farm workers' residential areas, exposure through clothing, and so forth.

In short, the realities of agricultural employment make it difficult if not impossible to meet the ideal conditions for establishment of pristine cholinesterase baselines. Many if not most agricultural workers, whether they be subjects in scientific experiments or simply presenting themselves for routine employment, are probably affected to some extent by a history of subacute exposure to organic phosphate residues. A realistic researcher therefore cannot ask "What is the 'true' baseline?" but can only ask "How much antecedent effect has there been?" "How can it best be estimated?" "How much is tolerable?" "How much is disqualifying?"

## III. Pre-Exposure Values

The University of California study proceeded pragmatically, with no special effort to recruit a crew with less than the ordinary chances of pre-experimental exposure. All subjects continued at their usual occupation of grape picking, right up to the commencement of the study: i.e., throughout a period of ten days during which three "pre-exposure" cholinesterase assays were made. During this period, the crew worked at two different ranches. Records showed that on one of them, Guthion had been applied 60 days before worker re-entry; on the other, Sevin and Dibrom had been applied six days before re-entry. Both waiting periods were adequate in terms of the state-of-the-art at that time; neither may have been, in the light of evidence developed during the University study itself.

"Pre-exposure" cholinesterase values may also have been affected by drift, home use of pesticides, and other factors mentioned previously. Whatever the reason, the results of the three "pre-exposure" cholinesterase tests demonstrate clearly that some of the subjects were not, in fact, in a pre-exposed condition, but were experiencing the effects of exposure to cholinesterase inhibiting agents before the study began. "Pre-exposure" values may be found in Table 1.

For purposes of brevity, this table and all the accompanying discussion have to do with plasma cholinesterase values. The discussion would be substantially the same if erythrocyte cholinesterase were substituted, since we are here concerned not so much with absolute values as with alternative analytical strategies. The first "pre-exposure" sample was drawn on a Monday, after subjects had not worked for a day or two. The second was drawn on Thursday of that same week. The third was drawn on Thursday of the following week.

Subjects began working in the experimental vineyard on Monday, October 5. In a moment, we shall comment on the timing of this sequence. As may be seen from Table 1, several participants had strikingly stable readings throughout the pre-exposure period. The values for Subject 11, for example varied by less than 4% during the 3 pre-exposure tests; the values for Subject 21, by less than 3%. But the plasma cholinesterase levels of other participants were equally striking for their instability. Some patterns assumed a "V" shape, with a first reading well within what is generally considered the normal range, a second reading quite low, and third reading as high or higher than the first. This was the case for example, with Subjects 16 and 17. The pre-exposure reports for Subjects 7, 13, 18, and others, followed an opposite pattern: an inverted "V", with a low initial reading, high second reading and low third reading, still other subjects, such as #10, had a steadily rising series of pre-exposure readings. Another pattern might plausibly have been found, although it was not in this particular sample: steadily declining

Table I

PRE-EXPOSURE PLASMA CHOLINESTERASE VALUES REPORTED
UNIVERSITY OF CALIFORNIA STUDY OF GRAPE
PICKERS, FRESNO COUNTY, 1970

| SUBJECT NUMBER | PRE-EXPOSURE VALUES | | | SUBJECT NUMBER | PRE-EXPOSURE VALUES | | |
|---|---|---|---|---|---|---|---|
| | First[1] | Second[2] | Third[3] | | First[1] | Second[2] | Third[3] |
| 1 | 2.02 | 5.11 | 2.25 | 13 | 2.58 | 3.55 | 2.51 |
| 2 | 3.83 | - | 2.68 | 14 | 2.89 | 2.68 | 2.51 |
| 3 | 2.81 | 3.07 | 3.22 | 15 | - | 2.64 | 2.53 |
| 4 | 2.39 | 2.30 | 2.53 | 16 | 2.97 | 1.39 | 3.26 |
| 5 | 2.07 | 2.01 | 2.20 | 17 | 2.81 | 1.54 | 2.85 |
| 6 | 1.86 | 2.30 | 1.86 | 18 | 2.12 | 3.65 | 2.29 |
| 7 | 1.84 | 3.99 | 2.11 | 19 | 2.39 | 2.68 | 2.46 |
| 8 | 2.67 | 2.64 | 2.39 | 20 | 2.16 | 2.95 | 2.25 |
| 9 | 2.76 | - | 2.94 | 21 | 2.35 | 2.44 | 2.43 |
| 10 | 2.44 | 3.88 | 4.24 | 22 | 2.76 | 3.02 | 3.31 |
| 11 | 2.55 | 2.49 | 2.38 | 23 | 3.49 | 3.93 | 4.09 |
| 12 | 2.36 | 2.59 | 2.62 | | | | |

[1] Blood drawn September 21

[2] Blood drawn September 24

[3] Blood drawn October 1

Note: Values expressed in pH stat units: i.e. µM/ml/min.

values throughout the pre-exposure period.

From among this tangle, the researcher must obviously accept a solution which falls well short of perfection. His task is to seek the approach which comes closest to validity for the largest number of participants. Following are some of the strategies which might be pursued in seeking the best approximation of a cholinesterase baseline among a group of agricultural workers.

A. "First day", "middle day" and "last day" strategies. The argument could conceivably be made that since none of the pre-tests was optimal for all subjects, one might as well take any of the three and trust that the individuals who were "high" on that day would be balanced by others who were "low". Such an arbitrary strategy has very little to recommend it. If any one of the three pre-test days were to be chosen over the others, it would most logically be the first, which was a Monday. Participants had not worked over the weekend, and their plasma cholinesterase levels presumably had a chance to rebound from whatever inhibition they may have sustained before the study began. The second and third pre-exposure blood specimens were drawn on Thursdays, when plasma cholinesterase levels could have been influenced by four working days of exposure.

Curiously enough, the average value for the Monday pre-test proved lowest of the three. The first of the Thursday readings was highest, and the final Thursday reading was intermediate. For the record, the respective averages were 2.55, 2.90 and 2.69, but none merits further discussion since there are much more satisfactory strategies available.

B. The "purist" strategy. One might in the interests of rigor, eliminate from further consideration all subjects whose pre-exposure readings did not follow a straight or nearly straight line. In studies of other populations, where cholinesterase stability may be the rule rather than the exception , this might be the strategy of choice. But the "purist" strategy has severe limitations in the world of agricultural labor, where cholinesterase stability is evidently the exception rather than the rule.

In the University of California study, elimination of subjects whose three pre-exposure values varied by more than ± 7.5% from the mean of such values, would result in elimination of 15 of 23 participants. To be sure, a study could be confined to eight subjects. But this would tend to lower the confidence we might have in changes observed during the post-exposure period, since statistical significance is a function, in part of sample size.

C. The "high-point" strategy. In view of the possibility of chronic marginal inhibition of cholinesterase among agricultural laborers generally, and in view of the difficulty of isolating them from continued exposure, it might be argued that the closest approximation to any individual farm worker's baseline must be the highest in his series of readings. If he were removed from further exposure for several weeks or months, his true baseline might well be even higher, but at least it would seem all lower readings could be rejected as having been depressed by artificial forcess--presumably organic phosphorus residues.

A major weakness in this strategy is that it makes no provision for the possibility of laboratory or clerical mistakes which err on the high side. In Table 1, for example, it seems unlikely that Subject 1 would have had pseudocholinesterase activity of 2.02 on September 21, that it would have vaulted to 5.11 on September 24, and fallen back to 2.25 on October 1. Plasma cholinesterase is more volatile than erythrocyte cholinesterase, but not that volatile. The reasonable interpretation is that 5.11 represents an error, and that the September 24 value was actually in the 2.00-2.25 range. Perhaps the true reading was 2.11, and the "2" was misread as a "5" by one of the clerks through whose typewriter the data passed.

Another objection may be raised against the "high-point" strategy. It appears, from animal experiments, that there may be one factor which produces an artificially elevated plasma cholinesterase level. It is the same factor which is better known for its cholinesterase-inhibiting properties--the very factor researchers are trying to hold constant in the pre-exposure period of such studies as these. Organophosphate compounds, it appears, produce a "rebound" effect during the recuperation period; for a time, an individual's plasma cholinesterase level may be higher than his true baseline.*[11,12,13,14]

If these two arguments against the "high-point" strategy are rejected, and this strategy is applied to the findings from the University of California study, it yields a mean pre-exposure value of 3.15 for the group as a whole.

D. The "averaging" strategy. Some observers feel that the fairest way to deal with variations in pre-exposure data is to take the average of the several readings. It might be argued that this has the effect of balancing laboratory errors on the high side against laboratory errors on the low side. By way of rebuttal, it might be argued that this strategy grossly admits into our baseline computations the effects of precisely the independent variable we should be trying to exclude. Subject 17, for example, had "pre-exposure" readings of 2.81, 1.54, and 2.85. If it is assumed that the first and third readings erred on the high side, and the second on the low side, and the average is struck, it proves to be 2.40--which is lower than this subject proved to have later in the study, after exposure to organic phosphorus residues. It seems much more plausible that this subject's baseline was in the vicinity of 2.80-2.90, and that the reading of 1.54 was the aberration.

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* This effect was noted, for example, by Durham and his colleagues in a study involving feeding DDVP to rats. After a sharp initial drop, and several weeks of fluctuation, pseudocholinesterase levels began to rise steadily until, after 10 weeks, they were more than 100% of normal.

By a similar token application of the "averaging" strategy to Subject 10 produces a very misleading result, which has the practical effect of minimizing change during the post-exposure phase of the study. This subject had readings of 2.44, 3.88, and 4.24. The average is 3.52. But it seems a reasonable interpretation that the subject was experiencing an artificially depressed level at the first reading, and that it had risen to something close to a true baseline by the time of the third reading. Indeed, even this interpretation may give the benefit of the doubt to the compounds involved, for we do not know how much higher this person's pre-exposure values might have risen if more readings had been taken.

If all such objections are rejected and the "averaging" technique is employed, it yields a mean of 2.72 for the pre-exposure portion of the University of California study: about 14% less than the "high-point" strategy.

E. The "no-wild-card" strategy. Some have suggested that when there is a variation between two of the pre-exposure readings, and the third, of 25% or more (20% or any other figure might be used as well), that the "wild card" reading be eliminated and the average of the other two be taken as the baseline. This has the advantage of ruling out distortions of extremely high or low readings, whether from laboratory error, organophosphate effect, or any other cause. The principal shortcoming of this strategy is its inability to cope meaningfully with cases in which two of the three readings are depressed. Applied to the subjects in the University of California study, it results in a group "pre-exposure" average of 2.66--among the lowest of any of the strategies outlined here.

F. The "control group" strategy. None of the foregoing strategies is above reproach, nor are any of the combinations and permutations which might be played on these same themes. If it is not feasible to keep farm workers away from all organic phosphate exposure for several weeks, perhaps baselines should derive from a control group which can be kept from all such exposure. This may be easier said than done. A control group well matched with the experimental group does not always come readily to hand. In some geographic areas, for example, it may be difficult to find able-bodied Mexican males who are not farm workers. And even in the case of non-farm subjects, there are possibilities for laboratory error and for depression of cholinesterase levels from diet, climate, and factors other than organophosphates.

Fortuitously enough, at almost exactly the time the University of California study was being conducted, other cholinesterase investigations were going on under different auspices[15] which made it possible to reconstruct a control group which closely matched the experimental group, with respect to age, sex, ethnic group and geographic area. In both the control and experimental groups, there were 18 men and 5 women. The average age of the men in the control group was 35, with a range from 19 to 55; in the experimental group, the men ranged in age from 18 to 59, with a mean of 33. The control group women averaged 27 years of age, with a range between 16 and 39; the women in the experimental group also averaged 27 years, with the range being from 17 to 45.

As we have seen in Table 1, study subjects received three pre-exposure cholinesterase tests, with the exception of Subject 15, who missed the first test, and Subjects 2 and 9, who missed the second. Participants in the control group received even more thorough screening. On the average, men in the control group had nearly four cholinesterase tests, women more than five. All these may be considered "pre-exposure" tests, since none of the controls had an occupation which involved any contact with pesticides or residues. Not only were farm workers excluded from the control group, but relatives of farm workers, and others who might have been exposed indirectly.

Members of both experimental and control groups lived in the same part of the San Joaquin Valley. Nearly 90% of the controls (20 of 23) were of Mexican ancestry, as were all the experimental subjects. Finally, and by no means least importantly, cholinesterase analysis in the two studies was done in the same laboratory by the same method.

Women in the control group had an average plasma cholinesterase level of 3.54; men, 3.78. Taking the control group as a whole, the mean was 3.73. This is considerably lower than has been reported in other studies of "normal populations,* but will be used here as the fairest possible approximation to a truly controlled cholinesterase value. It would be unfair to use control values from groups unmatched demographically, since there is every reason to believe that cholinesterase activity varies with sex, age, and climate.

It is instructive to recall that the highest pre-exposure average obtainable from the experimental group itself--through the "high-point" strategy--was 3.15. This figure, compared with 3.73 for a carefully matched control group, underscores the root methodological problem in any study of the effects of pesticides upon agricultural workers: before such a study even begins, the average farm worker has already sustained the effects of chronic, low-level exposure to organic phosphate residues. At the present time, no one knows how long it would take these depressed levels to find their true norm, or how high that norm would be if farm laborers were insulated from any further exposure. Unless and until such research is performed, perhaps the best alternative is to use baseline data from a scrupulously matched control group.

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* For example, Nabb and Whitfield, op. cit., reported a mean plasma cholinesterase value of 4.9 with a standard error of 0.02, among 18 normal subjects.

## IV. Post-Exposure Values

Exposure began early in the morning, Monday, October 5. Participants worked all that day and in the evening performed their usual ablutions, changed clothes, and so forth.* The first post-exposure blood samples were drawn after several hours of work on the second day: Tuesday, October 6. The second post-exposure blood test was taken on Thursday of that same week; the third, on Monday of the following week.

Hindsight enables us to perceive several respects in which the timing of this sequence was less than ideal. The final pre-exposure test was made on October 1, five full days before exposure began. It would have been preferable to take the last pre-exposure sample as close as possible to the beginning of exposure.

Secondly, data from this study strongly suggest that the first post-exposure test should be made no later than noon of the very day that exposure begins. As we shall see in a moment, several participants had severely depressed cholinesterase levels by the following morning. The study design should have permitted an even earlier identification of these individuals and referral to a physician's care.

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* No attempt was made in this study to influence or gather information about personal hygiene. Since the mean maximum temperature during the time the workers were in the test vineyard was 85.3°, and wine grape harvesting is an exceedingly dirty and gruelling job, it is a reasonable assumption that all participants bathed and changed clothing each evening.

Thirdly, the final post-exposure reading was taken at a time when it was beclouded by unknowns. The last day of work in the test vineyard was Friday, October 9. The subjects did not work on Saturday or Sunday, and on Monday, October 12, began cutting grapes in a new vineyard. The third blood test was taken after they had worked several hours in this location. Nothing seems to be known about the spray history of this new vineyard, and one cannot rule out the possibility that subjects may have been exposed to organic phosphorus residues.

On the other hand, one cannot rule out the possibility that a weekend at home, presumably without exposure, produced a regeneration of plasma cholinesterase. If the third post-exposure reading had been taken at the conclusion of work, Friday, October 9, it would have been a much more valid indicator. These methodological limitations should be kept in mind throughout the following discussion of post-exposure data.

A comparison of tables 1 and 2 reveals that eighteen of 23 participants experienced a decrease in plasma cholinesterase activity between the last pre-exposure test and the first post-exposure test. The average declined from 2.69 to 2.04, a difference of 24.2%. Fourteen subjects underwent a decrease during the two days between the first and second post-exposure tests; the study group as a whole showed an additional 4.4% decline, to a mean of 1.95. After another four days, including a weekend of non-exposure, nine participants manifested a decrease between the second and third post-exposure readings. The average for the entire group went down another 4.6%, to 1.86. We do not know how much lower the average might have gone if the group had continued to work in the test vineyard, but it is apparent that the rate of decrease was slackening; in a few more days, the

Table II

## POST-EXPOSURE PLASMA CHOLINESTERASE VALUES REPORTED UNIVERSITY OF CALIFORNIA STUDY OF GRAPE PICKERS, FRESNO COUNTY, 1970

| SUBJECT NUMBER | PRE-EXPOSURE VALUES | | | SUBJECT NUMBER | PRE-EXPOSURE VALUES | | |
|---|---|---|---|---|---|---|---|
| | First[1] | Second[2] | Third[3] | | First[1] | Second[2] | Third[3] |
| 1 | 0.82 | - | 1.02 | 13 | 0.82 | 1.49 | 1.83 |
| 2 | 1.87 | 1.91 | 1.84 | 14 | 2.03 | 1.87 | 1.98 |
| 3 | 1.79 | 1.57 | 2.43 | 15 | 1.44 | 2.93 | 1.75 |
| 4 | 1.23 | 1.57 | 1.88 | 16 | 2.17 | 2.47 | 1.96 |
| 5 | 1.57 | 1.10 | 1.43 | 17 | 2.87 | 2.66 | 3.08 |
| 6 | 1.83 | 1.58 | 2.23 | 18 | 3.56 | 1.61 | 1.82 |
| 7 | 1.60 | 1.36 | 1.24 | 19 | 1.94 | 1.15 | 1.88 |
| 8 | 2.46 | 1.57 | 1.67 | 20 | 1.92 | 2.17 | 1.52 |
| 9 | 2.29 | 1.87 | 1.24 | 21 | 2.87 | 1.36 | 1.78 |
| 10 | 3.21 | 2.89 | 2.12 | 22 | 2.70 | 1.70 | 2.12 |
| 11 | 0.45 | 1.44 | 1.57 | 23 | 4.55 | 3.45 | 3.10 |
| 12 | 0.82 | 3.19 | 1.24 | | | | |

[1] Blood drawn October 6

[2] Blood drawn October 8

[3] Blood drawn October 12

Note: Values expressed in pH stat units: µM/ml/min.

"rebound effect" might have begun.

The curve of post-exposure averages is much more regular than that of pre-exposure averages. Nevertheless, some of the same uncertainties must be conjured with: possible laboratory error, possible diurnal variation, etc., and the same basic question arises: from among the several possible alternatives, which is the best approach to measuring post-exposure effect? Following are some of the options.

A. The "first day" strategy. The greatest depression, proportionately and absolutely, occurs during the first day of exposure. A rational case could be made for confining the post-exposure analysis to this day alone. If a statistically significant amount of cholinesterase inhibition has taken place, the researcher knows all that he really needs to know. He knows, first of all, that the re-entry period being tested is not adequate to protect workers. He knows, secondly, that participants are in danger, and should be removed from further exposure. Contrariwise, if a statistically significant amount of cholinesterase inhibition has not taken place during the first day, it is doubtful that it will take place in succeeding days. In samples of the size we are considering here, changes on the order of 1% or 2% a day are not likely to prove significant by any of the standard statistical tests.

B. The "low point" strategy. In this approach, each subject would be considered as a discrete case. The rationale is that individuals differ in the amount of exposure they have actually received, since they work at different speeds, in different parts of the vineyard, etc. They differ in sensitivity to organophosphate residues. They differ also in their ability to regenerate cholinesterase and pseudocholinesterase. The best indicator of effect among some persons may be the first reading (this seems to have been the case with Subjects 1, 4, 11, 12, 13, 15), among others the second reading (see, for example, Subjects 3, 5, 6, 17, 18, 19, 21, 22), and among still others the third reading (see, e.g., Subjects 7, 9, 10, 16, 20, 23). In short, group averages may conceal more than they reveal.

C. The "averaging" strategy. In a 1970 study conducted by a leading pesticide manufacturer, this was in fact the technique employed. Its only possible justification is that it balances laboratory errors and diurnal variation on the high side against errors and "normal" variation on the low side. But this rationale is quite overwhelmed by the fact that averaging in the post-exposure period (unlike the pre-exposure period) destroys the very nature of the phenomenon under investigation. In the pre-exposure period, cholinesterase activity is supposed to be relatively stable. But following exposure, it becomes a dynamic, curvilinear phenomenon. The whole point of studies such as this is to evaluate the steepness of the curve.

D. The "last day" strategy. This is the antithesis of the "first day" strategy, the argument here being that the main problem among agricultural field laborers is not sudden acute exposure, and its dramatic effects, but subacute exposure and its insidious effect of a gradual decline in cholinesterase activity. On any given day, the amount of cholinesterase inhibition might be statistically insignificant--in fact, due to sampling error and diurnal variation, levels might appear to have risen--but if the study is continued long enough, daily fluctuations which are by themselves insignificant may add up to be meaningful indeed. According to this view, the most valid methodology is to keep drawing blood samples until it is certain that average values are going no lower and to compare the lowest average obtained with the best estimate of a baseline.

## V. Application of Various Strategies

There is not space here to pursue all the combinations of pre-exposure and post-exposure strategies, but for purposes of illustration let us examine the consequences of a few of the more reasonable combinations.

A. "Straight-line" pre-exposure and "last-day_ post exposure.

If a limitation of 5% variation around the average of each participant's three pre-exposure readings is made prerequisite to inclusion in the study, only two subjects qualify. As chance would have it, these two persons (Subjects 11 and 21) had pre- posure values so nearly comparable, and post-exposure declines so substantial, that under the Student or "t" test, the changes were very nearly significant at the 5% level. Obviously, however, more cases are usually necessary for the drawing of any kind of statistical conclusions. If the "purist" strategy accepts a variation up to ± 7.5% around each individual's pre-exposure average, eight subjects qualify. Combining this approach with the "last-day" post-exposure strategy yields the results on Table 3.

Table III

PLASMA CHOLINESTERASE VALUES WHEN "STRAIGHT-LINE" STRATEGY USED IN CALCULATION OF BASELINES[1] AND "LAST-DAY" STRATEGY USED FOR POST-EXPOSURE VALUES, UNIVERSITY OF CALIFORNIA STUDY OF GRAPE PICKERS, FRESNO COUNTY, 1970

| SUBJECT | PRE-EXPOSURE | POST-EXPOSURE | CHANGE | |
|---|---|---|---|---|
| | | | Number | Percent |
| 3 | 3.03 | 2.43 | - .60 | -19.8 |
| 4 | 2.40 | 1.88 | - .52 | -21.7 |
| 5 | 2.09 | 1.43 | - .66 | -31.6 |
| 8 | 2.57 | 1.67 | - .90 | -35.0 |
| 11 | 2.47 | 1.57 | - .90 | -36.4 |
| 12 | 2.52 | 1.24 | -1.28 | -50.8 |
| 14 | 2.69 | 1.98 | - .71 | -26.4 |
| 21 | 2.41 | 1.78 | - .63 | -26.1 |
| Average | 2.52 | 1.75 | - .78 | 31.0 |

[1] Subjects omitted if any of three pre-exposure values varied by more than ± 7.5% of pre-exposure average.

Application of the "Student" test to these data yields a "t" of 9.176 which in a one-tailed distribution with seven degrees of freedom, such as this is significant well beyond the .001 level of confidence

The "purist" pre-exposure strategy does not necessarily commend itself to us above all others, but because it restricts the sample size to small, manageable numbers let us use it as a referent against which to demonstrate the consequences of other post-exposure strategies.

B. "Straight-line" pre-exposure and "low-point" post-exposure.

The combination of these two strategies produces the following results.

Table IV

PLASMA CHOLINESTERASE VALUES WHEN "STRAIGHT-LINE" STRATEGY USED IN CALCULATION OF BASELINE[1] AND "LOW-POINT" STRATEGY USED FOR POST-EXPOSURE VALUES , UNIVERSITY OF CALIFORNIA STUDY OF GRAPE PICKERS, FRESNO COUNTY, 1970

| SUBJECT | PRE-EXPOSURE | POST-EXPOSURE | CHANGE | PERCENT |
|---|---|---|---|---|
| 3 | 3.03 | 1.57 | -1.46 | -48.2 |
| 4 | 2.40 | 1.23 | -1.17 | -48.8 |
| 5 | 2.09 | 1.10 | - .99 | -47.4 |
| 8 | 2.57 | 1.57 | -1.00 | -38.9 |
| 11 | 2.47 | 0.45 | -2.02 | -81.8 |
| 12 | 2.52 | 0.82 | -1.70 | -67.5 |
| 14 | 2.69 | 1.87 | - .82 | -30.5 |
| 21 | 2.41 | 1.36 | -1.05 | -43.6 |
| Average | 2.52 | 1.25 | -1.27 | -50.4 |

[1] Subjects omitted if any of three pre-exposure values vary by more than ± 7.5% of pre-exposure average.

Here, "t" proves to be 8.699, which is once again significant beyond the .001% level of confidence. Incidentally, a characteristic of all such statistical tests may be observed at this point. They are dependent not only on the total extent of change, but on the stability of the change. Virtually by definition, the "low-point" post-exposure strategy maximizes the extent of cholinesterase depression ; on average, the subjects showed a loss of 50.4% by this method, compared with 31.0% when the "last-day" strategy was employed. But the "last-day" approach proved the more significant statistically because there was less variation around the average.

C. "Straight-line" pre-exposure strategy and "averaging" post-exposure strategy.

As indicated a moment ago, the averaging of post-exposure values has little justification. It almost willfully camouflages the extent of post-exposure changes and in practical terms would have the effect of making a cholinesterase-inhibiting compound appear less hazardous than it actually is. However, in the present study post-exposure shifts were so great that even the "averaging" strategy could not disguise them.

D. "Straight-line" pre-exposure strategy and "first-day" post-exposure strategy.

The fourth and last of the post-exposure strategies discussed above rested upon the proposition that plasma cholinesterase is so volatile that all we really need to know, in studies such as this, is how it behaves in the first day of exposure. This theory, combined with the "purist" or "straight-line" pre-exposure strategy, produced

Table V

PLASMA CHOLINESTERASE VALUES WHEN "STRAIGHT-LINE" STRATEGY USED IN CALCULATION OF BASELINES[1] AND "AVERAGING" STRATEGY USED FOR POST-EXPOSURE VALUES, UNIVERSITY OF CALIFORNIA STUDY OF GRAPE PICKERS, FRESNO COUNTY, 1970

| SUBJECT | PRE-EXPOSURE | POST-EXPOSURE | CHANGE | PERCENT |
|---|---|---|---|---|
| 3 | 3.03 | 1.93 | -1.10 | -36.3 |
| 4 | 2.40 | 1.56 | - .84 | -35.0 |
| 5 | 2.09 | 1.37 | - .72 | -34.4 |
| 8 | 2.57 | 1.90 | - .67 | -26.1 |
| 11 | 2.47 | 1.15 | -1.32 | -53.4 |
| 12 | 2.52 | 1.48 | -1.04 | -41.3 |
| 14 | 2.69 | 1.96 | - .73 | -27.1 |
| 21 | 2.41 | 2.00 | - .41 | -17.0 |
| Average | 2.52 | 1.67 | - .85 | -33.7 |

[1] Subjects retained only if variation ≤ ± 7.5% around pre-exposure average.

Here "t" was 8.416, meaning that the changes were still significant at .001 level: there was less than one chance in a thousand that the results could have been a sampling artifact.

the results are found in Table VI.

This distribution is instructive primarily for what it reveals of the impact of one "outlying" case in a small series. Taking the eight cases as a whole, the percent change from pre-exposure to post-exposure was greater than that observed in either Table III or Table V. But Subject 21 whose reading,for one reason or another, was elevated rather than depressed after the first day of exposure, produced a sizeable standard deviation in the series, with the result that "t" was 4.015 and the confidence level was "only" .01.

E. "High-point" pre-exposure strategy and "last-day" post-exposure strategy. None of the foregoing should be taken as an endorsement of the "straight-line" pre-exposure strategy. The presentation is merely for illustrative purposes. Let us now examine some of the consequences if other pre-exposure strategies are followed. We shall employ throughout the following discussion a common post-exposure strategy--namely, the "last-day" approach but this, again, is done to facilitate internal comparisons, and should not be taken to mean that it is necessarily the preferable post-exposure strategy.

In Table VII there is no attempt to limit participants by any statistical criterion, as was the case in Tables III - VI. The entire series of 23 subjects appears in this and the following three tables. The larger sample, coupled with the fact that all but one of the subjects showed a decline in the variable under investigation, greatly strengthens the power of the "Student" test. The change is significant well beyond the highest confidence limits included in standard statistical tables. By extrapolation, we may

Table VI

PLASMA CHOLINESTERASE VALUES WHEN "STRAIGHT-LINE" STRATEGY USED IN CALCULATION OF BASELINES[1] AND "FIRST DAY" STRATEGY USED FOR POST-EXPOSURE VALUES, UNIVERSITY OF CALIFORNIA STUDY OF GRAPE PICKERS, FRESNO COUNTY, 1970

| SUBJECT | PRE-EXPOSURE | POST-EXPOSURE | CHANGE | PERCENT |
|---|---|---|---|---|
| 3 | 3.03 | 1.79 | -1.24 | -40.9 |
| 4 | 2.40 | 1.23 | -1.17 | -48.8 |
| 5 | 2.09 | 1.57 | - .52 | -24.9 |
| 8 | 2.57 | 2.46 | - .11 | -42.8 |
| 11 | 2.47 | 0.45 | -2.02 | -81.8 |
| 12 | 2.52 | 0.82 | -1.70 | -67.5 |
| 14 | 2.69 | 2.03 | - .66 | -24.5 |
| 21 | 2.41 | 2.87 | + .46 | +19.1 |
| Average | 2.52 | 1.65 | - .87 | -34.5 |

[1] Subjects limited to those in which variation ≤ ± 7.5% of pre-exposure average.

safely conclude that the differences between pre- and post-exposure values, as expressed in Table VII, are significant at the .000001 level.

F. "Averaging" pre-exposure strategy and "last-day" post-exposure strategy. When each participant's pre-exposure average is used as his baseline against which to compare his final post-exposure reading, results are shown on Table VIII.

As might have been expected, the percentage of change was smaller, using this technique, than under the "high-point" pre-exposure strategy. In two subjects, cholinesterase activity appeared actually to increase. Nonetheless, the general tendency was sufficiently consistent that these data are significant at the .00001 level of confidence: far beyond any customary standards of proof.

G. "No-wild-card" pre-exposure strategy and "last-day" post-exposure strategy. This pre-exposure strategy, it will be recalled, eliminates from consideration all values which deviate by more than 20% from the average of the individual's two other pre-exposure values. The assumption is made here that diurnal variations on the order of 20% are "normal", but that fluctuations of more than that are probably either laboratory error or the result of exposure to organic phosphates during the pre-test period.

This strategy may be considered a more sophisticated version of the "straight-line" strategy. It eliminates the distortions of dubious readings without eliminating the participant from the study altogether. Combining the "no-wild card" strategy with the "final day" post-exposure strategy yields the results to be found in Table IX.

Table VII

PLASMA CHOLINESTERASE VALUES WHEN HIGHEST PRE-EXPOSURE READING USED AS BASELINE AND FINAL READING USED AS POST-EXPOSURE VALUE, UNIVERSITY OF CALIFORNIA STUDY OF GRAPE PICKERS, FRESNO COUNTY, 1970

| SUBJECT | HIGHEST PRE-EXPOSURE VALUE | FINAL POST EXPOSURE VALUE | CHANGE | | SUBJECT | HIGHEST PRE-EXPOSURE VALUE | FINAL POST EXPOSURE VALUE | CHANGE | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Number | Percent | | | | Number | Percent |
| 1 | 5.11 | 1.02 | -4.09 | -80.0 | 13 | 3.55 | 1.83 | -1.72 | -48.5 |
| 2 | 3.83 | 1.84 | -1.99 | -52.0 | 14 | 2.89 | 1.98 | - .89 | -30.8 |
| 3 | 3.22 | 2.43 | - .79 | -24.5 | 15 | 2.64 | 1.75 | - .89 | -33.7 |
| 4 | 2.53 | 1.88 | - .65 | -25.7 | 16 | 3.26 | 1.96 | -1.30 | -39.9 |
| 5 | 2.20 | 1.43 | - .77 | -35.0 | 17 | 2.85 | 3.08 | + .23 | + 8.1 |
| 6 | 2.30 | 2.23 | - .07 | - 3.0 | 18 | 3.65 | 1.82 | -1.83 | -50.1 |
| 7 | 3.99 | 1.24 | -2.75 | -68.9 | 19 | 2.68 | 1.88 | - .80 | -29.9 |
| 8 | 2.67 | 1.67 | -1.00 | -37.5 | 20 | 2.95 | 1.52 | -1.43 | -48.5 |
| 9 | 2.94 | 1.24 | -1.70 | -57.2 | 21 | 2.44 | 1.78 | - .66 | -27.0 |
| 10 | 4.24 | 2.12 | -2.12 | -50.0 | 22 | 3.31 | 2.12 | -1.19 | -36.0 |
| 11 | 2.55 | 1.57 | - .98 | -38.4 | 23 | 4.09 | 3.10 | - .99 | -24.2 |
| 12 | 2.62 | 1.24 | -1.38 | -52.7 | Average | 3.15 | 1.86 | -1.29 | -41.0 |

Table VIII

PLASMA CHOLINESTERASE VALUES WHEN INDIVIDUAL PRE-EXPOSURE AVERAGE USED AS BASELINES AND FINAL READING USED AS POST-EXPOSURE VALUE, UNIVERSITY OF CALIFORNIA STUDY OF GRAPE PICKERS, FRESNO COUNTY, 1970

| PARTICIPANT | PRE-EXPOSURE AVERAGE | FINAL POST-EXPOSURE | CHANGE | | PARTICIPANT | PRE-EXPOSURE AVERAGE | FINAL POST-EXPOSURE | CHANGE | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Number | Percent | | | | Number | Percent |
| 1 | 3.13 | 1.02 | -2.11 | -67.1 | 13 | 2.88 | 1.83 | -1.05 | -36.5 |
| 2 | 3.25 | 1.84 | -1.41 | -43.3 | 14 | 2.69 | 1.98 | - .71 | -26.4 |
| 3 | 3.03 | 2.43 | - .60 | -19.8 | 15 | 2.59 | 1.75 | - .84 | -32.4 |
| 4 | 2.40 | 1.88 | - .52 | -21.7 | 16 | 2.54 | 1.96 | - .58 | -22.8 |
| 5 | 2.09 | 1.43 | - .66 | -31.6 | 17 | 2.40 | 3.08 | + .68 | +28.3 |
| 6 | 2.01 | 2.23 | + .22 | +10.9 | 18 | 2.69 | 1.82 | - .87 | -32.3 |
| 7 | 2.64 | 1.24 | -1.40 | -53.0 | 19 | 2.51 | 1.88 | - .63 | -25.1 |
| 8 | 2.57 | 1.67 | - .90 | -35.0 | 20 | 2.45 | 1.52 | - .93 | -38.0 |
| 9 | 2.85 | 1.24 | -1.61 | -56.5 | 21 | 2.41 | 1.78 | - .63 | -26.1 |
| 10 | 3.52 | 2.12 | -1.40 | -39.8 | 22 | 3.03 | 2.12 | - .91 | -30.0 |
| 11 | 2.47 | 1.57 | - .90 | -36.4 | 23 | 3.84 | 3.10 | - .74 | -19.3 |
| 12 | 2.52 | 1.24 | -1.28 | -50.8 | Average | 2.72 | 1.86 | - .86 | -31.6 |

## Table IX

## PLASMA CHOLINESTERASE VALUES WHEN "NO-WILD-CARD" STRATEGY[1] USED IN CALCULATION OF BASELINES AND FINAL READING USED AS POST-EXPOSURE VALUE , UNIVERSITY OF CALIFORNIA GRAPE PICKER STUDY, FRESNO COUNTY, 1970

| SUBJECT | BASELINE | FINAL POST-EXPOSURE | CHANGE | | SUBJECT | BASELINE | FINAL POST-EXPOSURE | CHANGE | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Number | Percent | | | | Number | Percent |
| 1 | 2.12 | 1.02 | -1.10 | -51.9 | 13 | 2.88 | 1.83 | -1.05 | -36.5 |
| 2 | 3.25 | 1.84 | -1.41 | -43.4 | 14 | 2.69 | 1.98 | - .71 | -26.4 |
| 3 | 3.03 | 2.43 | - .60 | -19.8 | 15 | 2.59 | 1.75 | - .84 | -32.4 |
| 4 | 2.40 | 1.88 | - .52 | -21.7 | 16 | 3.12 | 1.96 | -1.16 | -37.2 |
| 5 | 2.09 | 1.43 | - .66 | -31.6 | 17 | 2.83 | 3.08 | + .25 | + 8.8 |
| 6 | 2.01 | 2.23 | + .22 | -10.9 | 18. | 2.20 | 1.82 | - .38 | -17.3 |
| 7 | 1.98 | 1.24 | - .74 | -37.4 | 19 | 2.51 | 1.88 | - .63 | -25.1 |
| 8 | 2.57 | 1.67 | - .90 | -35.0 | 20 | 2.45 | 1.52 | - .93 | -38.0 |
| 9 | 2.85 | 1.24 | -1.61 | -56.5 | 21 | 2.41 | 1.78 | - .63 | -26.1 |
| 10 | 4.06 | 2.12 | -1.94 | -47.8 | 22 | 3.03 | 2.12 | - .91 | -30.0 |
| 11 | 2.47 | 1.57 | - .90 | -36.4 | 23 | 3.84 | 3.10 | - .74 | -19.3 |
| 12 | 2.52 | 1.24 | -1.28 | -50.8 | Average | 2.69 | 1.86 | - .83 | -30.8 |

[1] If any of the individual's three pre-exposure values departs from the average of his other two values by more than 20.0% it is dropped, and the average of the remaining two is used as his baseline. Otherwise, his baseline consists of the average of all three of his pre-exposure reading.

This technique results in the elimination of several very high pre-exposure values which are probably laboratory errors. This, in turn, results in a lessening of the drop between pre-and post-exposure values for those individuals, and accounts for the fact that the decrease for the group as a whole is slightly less than in the previous two strategies. But, still, "t" is 8.14, which in a single-tailed test with 22 degrees of freedom is significant at an extraordinary level of confidence.

H. "Control group" pre-exposure strategy and last-day" post exposure strategy.

As noted earlier, no matter how we may manipulate the pre-exposure values, insofar as a study group consists of bona fide agricultural laborers, we cannot be certain that they are free from exposure during a "pre-exposure" period. Indeed, it would be closer to the mark to say that we can be almost certain they are not completely free from the effects of occupational exposure to organophosphorus residues. Under these circumstances, every one of the strategies illustrated in Tables III - IX understates the extent to which cholinesterase levels would have been reduced if truly uncontaminated baselines were ascertainable.

If it is unfeasible to isolate farm workers during the weeks or months that might be required for a return to true baselines, perhaps most valid alternative is to use baselines from another group, which we know to be free from organic phosphate exposure, and which is matched with the experimental group for every variable under our control. Table X shows the results when final post-exposure values from the

Table X

PLASMA CHOLINESTERASE VALUES WHEN CONTROL GROUP AVERAGES USED AS BASELINE AND FINAL READING USED AS POST EXPOSURE VALUE , UNIVERSITY OF CALIFORNIA STUDY OF GRAPE PICKERS, FRESNO COUNTY, 1970

| SUB-JECT | SEX | CON-TROL GROUP AVER-AGE | FINAL POST-EXPO-SURE | CHANGE | | SUB-JECT | SEX | CON-TROL GROUP AVER-AGE | FINAL POST EXPO-SURE | CHANGE | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Number | Percent | | | | | Number | Percent |
| 1 | M | 3.78 | 1.02 | -2.76 | -73.0 | 13 | M | 3.78 | 1.83 | -1.95 | -51.6 |
| 2 | M | 3.78 | 1.84 | -1.94 | -51.3 | 14 | M | 3.78 | 1.98 | -1.80 | -47.6 |
| 3 | M | 3.78 | 2.43 | -1.35 | -35.7 | 15 | M | 3.78 | 1.75 | -2.03 | -53.7 |
| 4 | M | 3.78 | 1.88 | -1.90 | -50.3 | 16 | M | 3.78 | 1.96 | -1.82 | -48.1 |
| 5 | M | 3.78 | 1.43 | -2.35 | -62.2 | 17 | F | 3.54 | 3.08 | - .46 | -13.0 |
| 6 | M | 3.78 | 2.73 | -1.55 | -41.0 | 18 | M | 3.78 | 1.82 | -1.96 | -51.9 |
| 7 | M | 3.78 | 1.24 | -2.54 | -67.2 | 19 | F | 3.54 | 1.88 | -1.66 | -46.9 |
| 8 | F | 3.54 | 1.67 | -1.97 | -55.6 | 20 | M | 3.78 | 1.52 | -2.26 | -59.8 |
| 9 | F | 3.54 | 1.24 | -2.30 | -65.0 | 21 | F | 3.54 | 1.78 | -1.76 | -49.7 |
| 10 | M | 3.78 | 2.12 | -1.66 | -43.9 | 22 | M | 3.78 | 2.12 | -1.66 | -43.9 |
| 11 | M | 3.78 | 1.57 | -2.21 | -58.5 | 23 | M | 3.78 | 3.10 | - .68 | -18.0 |
| 12 | M | 3.78 | 1.24 | -2.54 | -67.2 | Aver-age | | 3.73 | 1.86 | - .87 | -50.1 |

experimental group are compared with baselines derived from a control group.

The control group was matched with the experimental group not only with respect to sex, as indicated in Table X, but with respect to age, ethnic group, and geographic area.

It might be argued that certain variables were uncontrolled: income, education, diet, housing, and so forth. A form of indirect control was achieved, through, by virtue of the fact that nearly all the members of both experimental and control groups were Spanish-speaking. In a rural California county, that means they were both drawn from essentially the same socio-economic stratum: in terms of W. Lloyd Warner's six-fold classification, it would be called the "lower-lower". Most of the persons in the control group were disabled or unemployed, and subsisting on welfare--which means that they had incomes comparable to those of farm workers, lived in the same kinds of neighborhoods, ate the same kinds of foods, etc.

In short, the only major difference between experimental and control groups, it would seem, was occupational: the former consisted of persons who, being farm workers, might have been occupationally exposed to pesticides before the study began; the latter consisted of persons who were either unemployed altogether, or had jobs (such as baby-sitting) which involved no contact with pesticides.

This approach, which has so much to recommend it logically, yields a "t" of 17.027--by far the highest of any technique we have reviewed. In other words, the "control group" strategy produces the most significant results, statistically speaking. There is literally less than one chance in a million that the data in Table X could have been obtained through the operation of chance factors.

## VI Conclusions and Recommendations

Many other combinations of "pre-exposure" and "post-exposure" strategies could be pursued if one desired: "no-wild-card" vs. "first-day"; "control group" vs. "low-point"; and so forth. There is no need to go into further possibilities here.

Tables III - X, and the accompanying discussion, will suffice to make the basic point: different strategies have different merits and demerits. Some may come close to being flatly "wrong", but none is flatly "right". The selection of a strategy by a researcher in the field of pesticides and farm worker health will depend ultimately on his point of view. If a researcher wishes to pay obeisance to the canons of scientific method but at the same time put the best possible face on the use of organic phosphorus pesticides, he will choose pre- and post-exposure strategies which have a levelling effect: the "no-wild-card" and "averaging" approaches, perhaps. It happens that in the University of California study, residues proved to be very hazardous even when these conservatizing strategies were employed. But in other studies, with less unambiguous results, the choice of these analytical techniques might make the difference between a pesticide re-entry period receiving a clean bill of health or not.

If, however, a researcher has a "bias" in the direction of health and safety, he would do well to select methods of analysis which lean in the other direction. He might wish, for example, to use the "control group" strategy and the "low-point" strategy for his pre- and post-exposure data, respectively.

If a researcher wishes to try to follow a line somewhere between the "pro-pesticide" and pro-health" positions, he has many possible compromises from which to choose. For example, the "straight-line" pre-test strategy and "first-day" post-exposure strategy together yielded results which were about "average".

Let there be no mistake: perfect neutrality in this field of investigation, as in many others, is a chimera. The researcher who claims he has no point of view, and really believes it, is in fact biased in the worst possible way. His ignorance of his own point of view renders him incapable of controlling it. The most "unbiased" thing an investigator in this field can do is to state clearly, at the outset, the array of analytical methods he might have chosen, the one he chose, and his reasons for choosing it. The reader may then judge for himself whether the choice was wise, and whether the study's conclusions would have been different, given different methodological choices.

Let there be no mistake on another point, too: the analytical strategy chosen will have far-reaching ramifications. The strategies reviewed in this paper, for example, yielded results which varied by more than 40%. If a study of this type is to be used as the basis for setting "safe waiting periods"--and that is presumably the purpose of all such studies--a variation of 40% could mean the difference between a re-entry period being set at 30 days, say, or 45 days.

The distinction is of far more than academic interest to agricultural workers, and to many other parties as well: growers, pesticide manufacturers and retailers, agricultural pest control operators, farm labor unions, public health agencies, practicing physicians, ecology groups, and perhaps eventually the American housewife.

It is quite conceivable that studies such as the one conducted by the University of California Agricultural Extension Service in 1970 will, in the last analysis, demonstrate that some fundamental assumptions about pest control simply cannot be reconciled with farm worker safety. If a "pro-health" analytical strategy is adopted, and a waiting period is set at 45 days instead of 30, it is entirely possible the accustomed goal of insect extermination cannot be achieved: a species which does not complete a cycle within four weeks may within six weeks.

It is by no means out of the question that the philosophy of "zero population growth" in agricultural pest control will have to be modified, and a certain amount of cosmetic damage by thrips on orange rinds, for example, will have to be accepted. In the ultimate analysis, perhaps, pesticide research rests not so much on the attitudes of manufacturers, growers, labor, and government as on attitudes of the American consumer.

A final word seems called for by the University of California data. By generally agreed upon medical standards (i.e., plasma cholinesterase inhibition of more than 20%), 13 of 23 subjects should have been removed from the study after the first day of exposure, and another six should have been removed on the basis of their second post-exposure test. In ethical terms, only four participants should have been allowed to continue working in the treated vineyard up to the time of the third and final post-exposure blood test.

Throughout this paper, we have used the phrase "pesticide research", but that is not strictly accurate. The better term would be "human research", in which pesticides happen to be the independent variable. It is incumbent upon anyone who engages in human research to acquaint himself with certain very exacting canons of research ethics, and to build them into his study design. One such canon is that danger to participants must be minimized. In future studies of the sort we have been discussing here, investigators must, at the very least, maintain constant surveillance for cholinesterase loss of more than 20%, and if it is found, the affected individuals must be immediately removed from further exposure and placed under medical supervision. Better yet, research should be designed in such a way that participants do not sustain clinically significant cholinesterase loss. In a series of 23 subjects, for example, an average drop in pseudocholinesterase of 10% would be quite sufficient to demonstrate that a safe waiting period had not yet been achieved. Perhaps the greatest single justification for biostatistics is that they enable a researcher to be "scientific" and ethical at the same time: changes which are statistically significant in a cohort as a whole usually take place before individual changes which are clinically dangerous, and make it possible to protect the human beings whose physiology is under investigation.

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